AN ANALYSIS OF RESPONSE TO OSMOTIC STRESS IN SELECTED DECAPOD CRUSTACEA

WARREN J. GROSS [1]

Department of Zoology, University of California, Los Angeles, California

Krogh (1939) and Prosser *et al.* (1950) have reviewed the subject of osmotic behavior in aquatic animals. Beadle (1943) has reviewed the importance of osmotic regulation in the evolutionary migration of marine animals to fresh water habitats. Pantin (1931) has discussed the origin of body fluids in animals. Robertson (1949, 1953) presents extensive information concerning ionic regulation among several groups of invertebrates. Höber *et al.* (1945) consider the physical chemistry involved in osmotic regulation. Jones (1941) showed that the crab *Pachygrapsus crassipes* regulates in dilute or concentrated sea water after 72 hours of immersion. However, osmotic regulation as a function of time for *Pachygrapsus* apparently has not been studied. This would seem to be an essential parameter in its ecologic importance, especially in the first few hours.

Salt and water pools have been suggested several times (Hukuda, 1932; Scholles, 1933; Beadle and Shaw, 1950; Gross, 1954.) The present investigation demonstrates that in the crabs studied, osmotic changes in the blood are brought about mainly by salt exchanges and not water. The presence of functional salt and water pools is considered.

Exoskeleton permeability is very unequal among decapods. Nagel (1934) found a correlation between regulating ability and permeability of the exoskeleton to applied sodium iodide in several crabs. The correlation is also established in the present study on the permeability for electrolytes and water, comparing six species of crabs and a crayfish.

The gills as seats of salt and water exchange and organs of regulation in crabs have been implicated mainly by eliminating other probable structures (Margaria, 1931; Nagel, 1934; Krogh, 1938). Webb (1940) produced histological evidence of a correlation in crabs between the ability to regulate and the complexity of the gills. Pieh (1936) demonstrated that isolated gills of regulating crabs show increased respiratory rates when exposed to osmotic stresses, thus suggesting increased work for regulation in these tissues. Koch *et al.* (1954) have produced direct evidence that *in vitro* the gills of *Eriocheir* can remove salts from a medium against a gradient. The work presented here offers direct evidence that the gill chamber of *Pachygrapsus* is a locus of electrolyte and water exchange, and that an osmotic gradient can be held in the chamber during an osmotic stress.

The energy expended for osmotic regulation has repeatedly been a subject of study by oxygen uptake determination. Schlieper (1929), Schwabe (1933) and Flemister and Flemister (1951) demonstrated that the metabolic rate increases when crabs are under osmotic stress. This they attributed to added osmotic work.

[1] Present address: Division of Life Sciences, University of California, Riverside.

Krogh (1939), Wikgren (1953) and Potts (1954) throw doubt on this interpretation.

The present study indicates that rates of oxygen consumption do not manifest increased osmotic work, but muscular activity.

Materials and Methods

The principal subjects for this investigation were three species of decapod Crustacea: (1) *Birgu latro* Linnaeus, the anomuran coconut crab, native of the Indo-Pacific region and an inhabitant of land, was collected on the island of Guam and maintained in the laboratory in Los Angeles. (2) *Emerita analoga* Rathbun, the common anomuran sand crab, found on sandy beaches burrowed in the sand near the level of the washing waves, was collected at Santa Monica and Corona Del Mar, California. (3) *Pachygrapsus crassipes* Randall, the brachyuran shore crab, found in high intertidal zones and in semi-terrestrial situations, was collected at Ballona Creek and Flat Rock Point, California.

The concentration of fluids was determined in two manners: (a) melting point method as described by Gross (1954), which permitted determinations on volumes as small as one $mm.^3$; (b) conductivity measurements using a 1000-cycle bridge. This allowed determinations on a two-ml. sample which was not necessarily expended but could be returned to the experimental vessel. Of course, this method measured only electrolytes. Units of resistance were converted to per cent of a standard sea water.

Oxygen consumption was measured by means of the Scholander-Wennesland respirometer as described in Wennesland (1951). All determinations were made at 16° C., a temperature to which the experimental animals were accustomed. Particular care was taken to assure that immersed animals were completely covered. No readings were made until the animal remained in the chamber for at least one hour; this was to allow them to become accustomed to the new environment.

Results

1. Osmotic Regulation as a Function of Time

Measurements of blood concentration during immersion in water were made throughout the range of water salinity, in which life could be sustained. The behavior of a species was determined partially from single readings on specimens exposed to certain stresses for given periods. However, regulation as a time function in individual specimens was followed over extended periods by two methods: (1) melting point determination on blood samples extracted periodically during immersion, and (2) periodic measurements of losses or gains of conductivity of the medium. A combination of these two methods was found best for studying individual responses over extended periods.

Emerita, a non-regulator

It was first established by melting point determinations that the body fluids of *Emerita* are isotonic to normal sea water (3.46% salt) and that this animal cannot sustain an osmotic gradient between its blood and external medium as a steady-

state condition. When three specimens were immersed in each of the following concentrations of sea water: 50, 75, 90, 110, 125, and 150% (total of 18 animals), their body fluids were isotonic to their respective external media within two hours or less after immersion. Thus *Emerita* shows no ability to regulate osmotically.

TABLE I

Solute space calculated from the relationship between concentration changes in the medium and the blood of the animal immersed in 5 times its volume of water

Specimen number	Medium (% sea water)	Change in blood (% sea water)	Change in medium (% sea water)	$\frac{\text{Change in blood}}{\text{Change in medium}}$	Calculated solute space (water equivalent) % body weight
			Emerita		
1	50	46.3	3.7	12.5	36
2	50	45.6	4.4	10.4	44
3	60	36.5	3.5	10.4	44
4	75	23.0	2.0	11.5	40
5	75	23.1	1.9	12.2	37
6	75	22.8	2.2	10.4	44
7	125	23.2	1.8	12.9	38
8	125	23.1	1.9	12.2	37
9	150	45.3	4.7	9.6	47
10	150	46.3	3.7	12.5	36
Mean				11.5	40
			Pachygrapsus		
1	25	22.6	2.7	8.4	50
1	25	15.3	1.9	8.1	51
2	50	13.7	2.0	6.9	60
3	39	13.2	1.8	7.3	57
4	50	14.7	1.9	7.8	53
5	50	14.2	1.8	7.9	53
6	50	9.5	1.3	7.3	57
7	125	10.5	1.4	7.5	56
8	145	7.9	1.0	7.9	53
9	150	15.8	2.0	7.9	53
Mean				7.7*	54

* $S = \frac{5/7.7}{1.2} \times 100 = 54\%$.

Individual specimens which had never been removed from normal sea water, were then immersed in dilute or concentrated media of 5 times the volume of the animal. Then the electrical resistance changes in the medium were followed until the resistance was stabilized and here the body fluids could be considered to be isotonic to the medium. The change in the blood concentration is therefore equal to the difference between the final concentration of the stress medium and the concentration of normal sea water. If, then, a constant ratio between osmotic changes in the blood and medium could be established, a conductivity variation in the medium

could be interpreted in terms of a change in the blood concentration, so that at any time the osmotic pressure of the blood could be estimated by such conductivity readings. Data in Table I demonstrate that such a ratio is relatively constant over a wide range of osmotic stresses, the mean value showing a change in the body fluids equivalent to 11.5% sea water for each 1% sea water change in the medium.

The rate of approaching equilibrium with the external environment suggests a physical, diffusion phenomenon. The curves are indicated in Figure 3. However, there are individual variations which result particularly with size, the smaller animals reaching equilibrium first.

Exploratory experiments suggested that *Callianassa affinis, Upogebia* sp., *Cancer antennarius, C. gracilis,* and *Pugettia producta* behave similarly.

Regulating forms immersed in water

Pachygrapsus in normal sea water is not necessarily isotonic to the medium. (Note the variation in initial blood concentration in Figure 1.) Specimens were immersed in stress media of five times their respective volumes and salinity changes in the medium were repeatedly noted by the conductivity bridge. After a significant change in the medium was observed, a melting point determination was made on the blood.

As with *Emerita,* a relatively constant ratio between conductivity change of the external medium and osmotic pressure change in the body fluids of the animal was demonstrated (Table I). On the average, a change in the external medium equivalent to 1% sea water meant a change in the blood equivalent to 7.7% sea water.

Therefore, if the initial or final blood concentration of *Pachygrapsus* were known, conductivity measurements of the medium could be converted to represent the approximate osmotic pressure of the blood.

The crabs were able to tolerate the small volumes of medium for long periods, if moved to a fresh medium of the same conductivity at least every three hours.

Over extended periods the rate of regulation in an individual specimen of *Pachygrapsus* could be estimated by converting changes in the medium to body fluid concentrations assuming the above mean ratio of change in blood to change in medium. Occasional checks were made by melting point determinations of the blood. Subsequent deviations did not exceed 10%. Such an error could not obscure a trend. When readings were not needed at close intervals, the specimen was placed in a large volume of the desired salinity and after an appropriate period a melting point determination was made on the blood. This was done on most of the late readings (*e.g.,* 72 hours). Figure 1 illustrates osmotic regulation in *Pachygrapsus* as a function of time. It should be pointed out that a small error is introduced by using the conductivity method, for when a change occurs in the medium, the osmotic gradient is consequently reduced. In the extreme case where a change in the blood was equivalent to 40% sea water, about a 5% error was effected. However, since the conductivity method was used for brief periods, not exceeding 12 hours, osmotic changes detected were small and the consequent errors caused by reducing the osmotic gradient were usually insignificant.

As shown in Figure 1, *Pachygrapsus* can regulate osmotically in both hypotonic and hypertonic media. This confirms the work of Jones (1941). However, while Jones showed this to be true after 72 hours, the present investigation shows that

regulation is established immediately, and is sustained perfectly by some specimens in moderate stresses for a few hours. Regulation then diminishes gradually until equilibrium is reached, usually within 24 hours. However, several plateaus and steps on the osmotic behavior curves may be produced before equilibrium is finally reached. Equilibrium in the case of *Pachygrapsus* does not mean that the body fluids are isotonic to the external medium. Rather, it means that the blood of the animal has reached a steady state with respect to osmotic pressure.

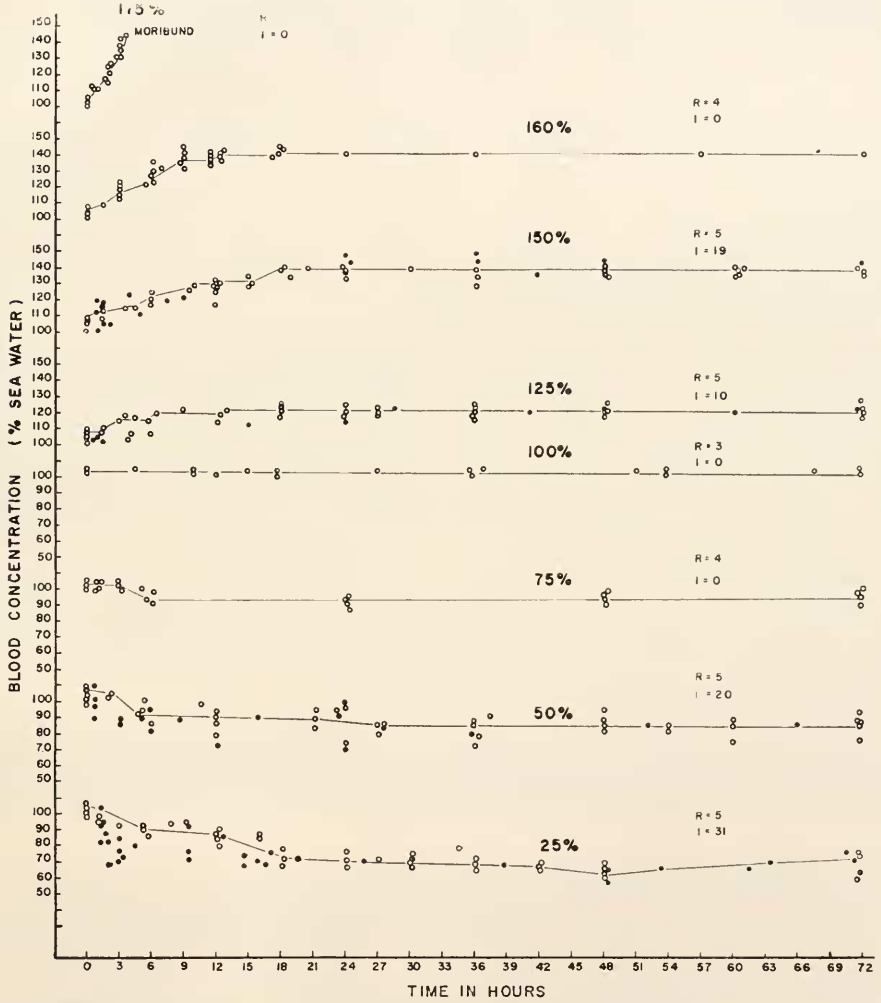

FIGURE 1. Osmotic regulation in *Pachygrapsus* as a function of time. "R" represents those specimens whose behavior was followed for extended periods by repeated determinations and is indicated by open points (○); "I" represents those specimens on which only one determination was made and is indicated by solid points (●). Solid line represents approximate median and is drawn through points representing the actual behavior of one specimen. All points are indicated unless coinciding with others. Concentration of the medium is indicated in per cent of normal sea water above the respective curve.

The plateaus and steps on the osmotic behavior curves reflect grading of the active regulatory processes or changes in accessory processes, *e.g.*, water movement over the gills. Only occasionally do fluctuations or dips occur. Such behavior is shown in Figure 1 by a crab immersed in 25% sea water.

A high degree of individual difference is demonstrated by the wide spread of points and the varying slopes among individual histories. Such variations could be caused by differences in size, age, sex, metabolic rate variances caused by physiological periodicities, *e.g.*, molting cycle, or external environment changes.

Concurring with Jones (1941), it was demonstrated that *Pachygrapsus* regulates better in hypotonic media than in hypertonic media. This phenomenon becomes evident from (a) the period of perfect regulation, (b) the slope of the regulation curve, (c) the total change in the concentration of the blood at equilibrium and (d) the extreme osmotic stresses endured by the crabs. These lines of evidence become apparent with inspection of Figure 1. However, it is worth mentioning that no animal lived in 175% sea water for as long as 6 hours. Yet several crabs actively survived 25% sea water for 72 hours. Prosser *et al.* (1955) demonstrated relatively strong tolerance to 170% sea water by *Pachygrapsus*. However, their experimental animals were gradually acclimated to lesser stresses before immersion in 170% sea water (personal communication).

There seems to be variation in the blood concentration of *Pachygrapsus* living in normal sea water. Jones (1941) reports that the body fluids of *Pachygrapsus* are hypotonic to normal sea water. Pearse (1931) found the body fluids of this crab hypertonic to normal sea water. As can be seen from Figure 1 the crabs used in the present investigation were usually hypertonic to their external medium when they were immersed in normal sea water. Such variations possibly can be explained by the fact that the osmotic pressure of the blood is a function of the molt cycle (Baumberger and Olmsted, 1928).

Token experiments suggested that *Uca* and *Hemigrapsus* regulate similarly to *Pachygrapsus*. Confirming the work of Jones (1941), *Uca* was found to regulate more strongly in hypertonic and hypotonic sea water than *Pachygrapsus*. In the salinity ranges from 50% sea water to 150% sea water, this form maintained almost perfect regulation for 36 hours.

While Jones (1941) found that *Hemigrapsus* could regulate strongly in dilute sea water, he was unable to show regulation in concentrated sea water after 72 hours immersion. The present investigation revealed that this crab can regulate up to 33% perfectly for 20 hours in 150% sea water.

Osmotic regulation in the land crab Birgus latro

The implications of osmotic regulation in the land crab *Birgus latro* have been discussed by Gross (1955). As mentioned there, these anomurans will drown when completely immersed for a day or so. It was therefore necessary to allow all the animals to rise slightly out of the water for exposure of their respiratory membranes to air. By this operation they partially released themselves from the imposed osmotic stresses. However, most of the external surface was immersed all of the time. Because of the limited number of specimens, only one specimen of *Birgus* could be studied in the representative stresses which were: 25, 50, 66 and 137% sea water, respectively (four specimens). In these cases the changes in the

osmotic pressure of the blood were followed by repeated melting point determinations on blood samples extracted at chosen times. Results are illustrated in Figure 2. These data show at least that *Birgus* is a strong regulator in both dilute and concentrated sea water. The moribund condition of the crabs after prolonged immersion in 25% and 137% sea water is not believed to be the direct result of the osmotic changes, since the two blood concentrations, specifically 70 and 119% sea water, are readily tolerated by this species (Gross, 1955). Anoxia seems to be a more satisfactory explanation for the moribund condition of these two specimens; however, high oxygen tensions failed to revive them. It is perhaps pertinent that

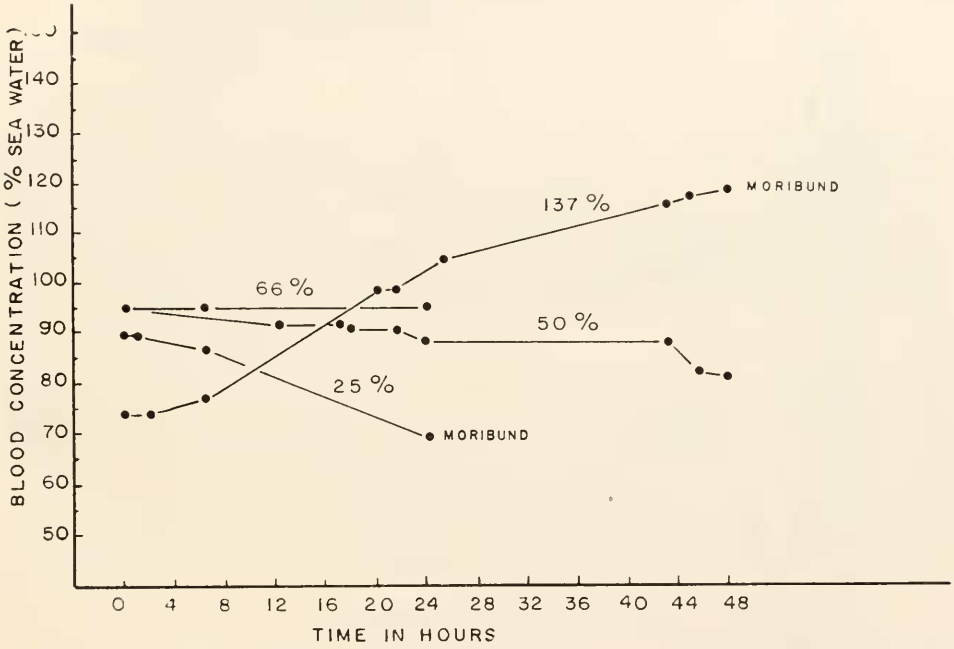

FIGURE 2. Osmotic regulation in *Birgus* as a function of time. Behavior of four specimens demonstrated by repeated melting point determinations on the blood. Concentrations of the medium are indicated in per cent of normal sea water over the respective curves.

the two animals exposed to the greatest stresses were most affected, but the fact that *Birgus* can regulate in concentrated sea water corroborates the observation that hypo-osmotic regulation is common among crabs showing some degree of terrestrial behavior (Jones, 1941; Gross, 1955).

Salt exchanges

When *Emerita* and *Pachygrapsus* are immersed in dilute or concentrated sea water, the osmotic pressure of their body fluids changes but corresponding weight changes under these conditions are small. In the case of *Emerita* a change in the body fluids equivalent to 25% sea water resulted in a weight change of less than 2% of the body weight. If pure water, this could effect a concentration change in the

blood of less than 6% on the assumption of 40% of the body weight being osmotically active water. This of course means that changes in the concentration of the body fluids are effected mostly by net changes in the solute content rather than water; 20% of the total concentration change of the blood was caused by water and 80% by solutes, in this case.

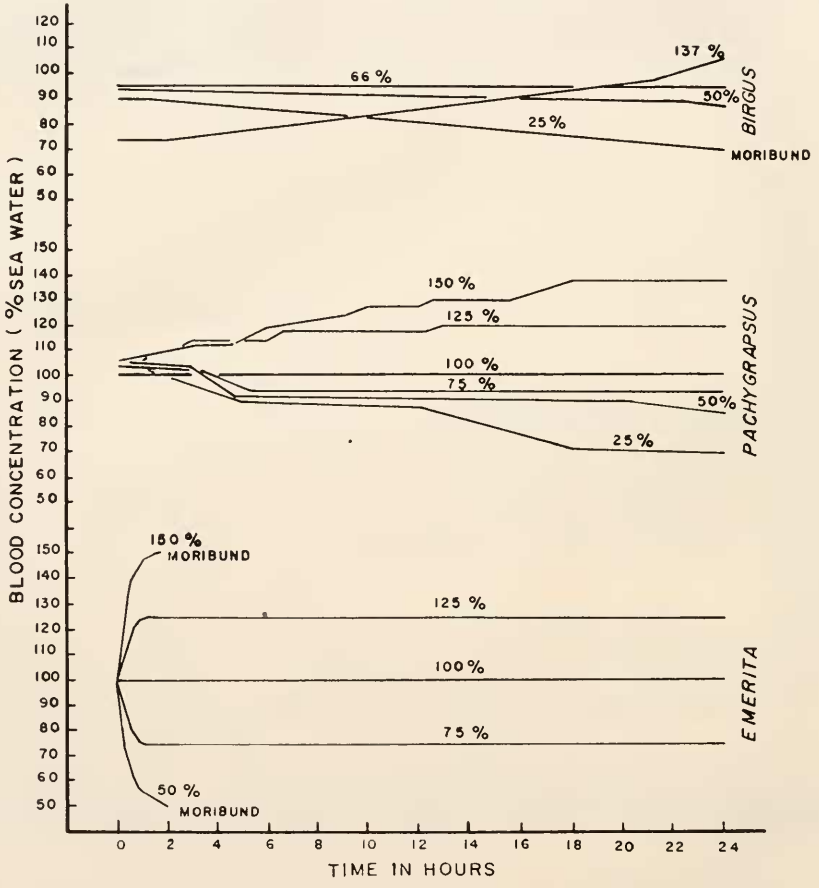

FIGURE 3. Comparative osmotic behavior of *Emerita, Pachygrapsus* and *Birgus*. Solid line indicates behavior of individual specimen which, in the case of *Emerita* and *Pachygrapsus,* represents the approximate average behavior of all individuals investigated for a given osmotic stress. All cases of *Birgus* are illustrated. Medium concentrations are indicated in per cent of normal sea water over the respective curves.

Curves for *Emerita* uncorrected for salt losses to the medium.

On the other hand, weight changes in *Pachygrapsus* during prolonged immersion in dilute and concentrated sea water deviating 75% from normal were so small they could hardly be considered significant. If water does cause changes in the blood concentration of these crabs, its effect is small. These findings are in accord with the work of Hukuda (1932).

Now the relatively persistent ratio between the concentration change in the blood and the external medium (Table I) suggests a method for estimating values for solute space which can be calculated from the equation:

$$S = \frac{v/p}{d} \times 100,$$

where S = solute space (equivalent in water) in per cent body weight,

$$v = \frac{\text{volume medium}}{\text{volume of specimen}},$$

$$p = \frac{\text{change in body fluids}}{\text{change in external medium}},$$

d = specific gravity of specimen.

Mean solute space values thus calculated were 54% body weight for *Pachygrapsus* and 40% body weight for *Emerita* (Table I). There was no evidence of a trend; that is, the values for solute space did not vary with different magnitudes of change in the osmotic pressure of the body fluids. Thus, if salt pools contribute to the osmo-regulatory mechanism they probably exert a constant effect over the range tested. That is, there is no varying degree of salt fixation or mobilization with increased osmotic stress, a phenomenon suggested by Hukuda (1932) and demonstrated by Gross (1954) in sipunculids.

It should be borne in mind that the above values for solute space are approximate and probably good only for the specific conditions of the experiment, for it is known that blood concentration and total water content change during certain phases of the molting cycle (Baumberger and Olmsted, 1928).

When solute space was estimated on two specimens of *Birgus*, values were 44 and 56% body weight, respectively, data which were subject to considerable error because changes in the external medium by evaporation could not be properly corrected.

2. The Osmo-Regulatory Mechanism

Jones (1941) and Prosser *et al.* (1955) have shown that the green glands of *Pachygrapsus* are ineffective as osmo-regulatory organs. Confirmatory evidence was established in the present investigation when the urine of 5 crabs immersed for 24 hours in 50% sea water was shown to be isotonic to the blood in all cases. Other regulatory mechanisms will be examined in the following section.

Osmotic regulation and permeability of the exoskeleton

Nagel (1934) demonstrated in several decapod Crustacea that non-regulators have more permeable exoskeletons than regulators. An attempt was therefore made to detect a correlation between the ability to regulate osmotically and the permeability of the exoskeleton in *Cambarus clarkii*, *Pachygrapsus*, *Hemigrapsus nudus*, *H. oregonensis*, *Cancer antennarius*, *C. gracilis* and *Pugettia producta*.

Since it was desired to know the role of exoskeleton in osmotic regulation of the different species, it was necessary to test the permeability of equal areas of exoskeleton of animals of about the same size. Discs of exoskeleton approximately three

cm. in diameter were removed from the carapaces of freshly killed animals. Each disc, hypodermis removed, was fitted against a rim of the end of a screw sleeve, then screwed into the end of a glass tube so that the chitinous disc formed the bottom surface (both inside and out) of the glass tube. The opening at the end of the screw sleeve determined the area of the chitin to be exposed, and was uniform for all cases. The tube was then filled with 10 ml. of normal sea water and the chitinous end was immersed in another tube containing 10 ml. of 50 per cent sea water. While the normal sea water inside the smaller tube simulated the body fluids of an animal, the dilute sea water in the larger tube simulated the external medium. The salt change in the dilute medium was then measured by a conductivity bridge after 24 hours to determine the relative permeability. As a check against leaks of water between the rim of the sleeve and disc, a few drops of concentrated dye were placed in the sea water and when such a leak occurred the dye appeared in the outside dilute medium. There was no intentional agitation and conditions for the different species were essentially uniform. Individuals chosen were apparently not close to molt.

Since the salt concentration in the dilute medium increased measurably in all cases, it can be said that the exoskeleton in all species studied is permeable either to salts or to water. If the exoskeleton were permeable only to water, then the volume of fluid in the tube which originally contained 100 per cent sea water should increase; this was never observed. The salinity changes through the exoskeleton of regulating crabs were small. However, if distilled water is used in the outside medium instead of 50 per cent sea water, a 5 per cent change can be effected in the concentration of the inner medium within 24 hours, using samples of *H. oregonensis*. This would afford a detectable volume change were semi-permeability the nature of the exoskeleton, but no volume change was observed. Thus it can be said that the exoskeleton of all non-regulators studied, and at least *Hemigrapsus oregonensis* among the regulators, is permeable to both salts and water. The average permeability of three samples of exoskeleton from each species is expressed in Figure 4. The values should be slightly higher than indicated because the osmotic gradient was reduced as salinity changes occurred. Such differences, however, would be insignificant for regulators.

It can be seen from these results that there is a correlation between regulating ability and exoskeleton permeability. The animal which probably endures the highest osmotic stress in nature, *Cambarus,* shows the lowest permeability value, although sufficient determinations were not made to state that this is significantly different from the values for *Pachygrapsus* and *H. nudus*. The non-regulator which demonstrates the lowest permeability, *C. gracilis,* still shows a value which is three times that of *H. oregonensis,* which has the highest permeability among the regulators.

Pachygrapsus is probably the best regulator among the crabs and it shows the lowest permeability value, but whether the correlation is this good cannot be said on the basis of the evidence available. Too few cases have been used to place weight on the apparent difference between *H. nudus* and *H. oregonensis*. The differences among the non-regulators do not seem to be adaptive, since these forms are stenohaline.

In order to determine whether or not the chitinous exoskeleton shows greater permeability in one direction, a section of the exoskeleton of *Pachygrapsus* was tested as above, first measuring the conductivity changes in the outer medium of

distilled water after four hours when the inner medium is normal sea water, then reversing the media, so that the sea water is on the outside, and measuring the salinity increase in the inner medium of distilled water after four hours. Permeability on three samples seemed approximately equal in both directions.

An interesting question arises as to whether regulating and non-regulating crabs differ in their active mechanisms. Could *Pachygrapsus,* for example, regulate in

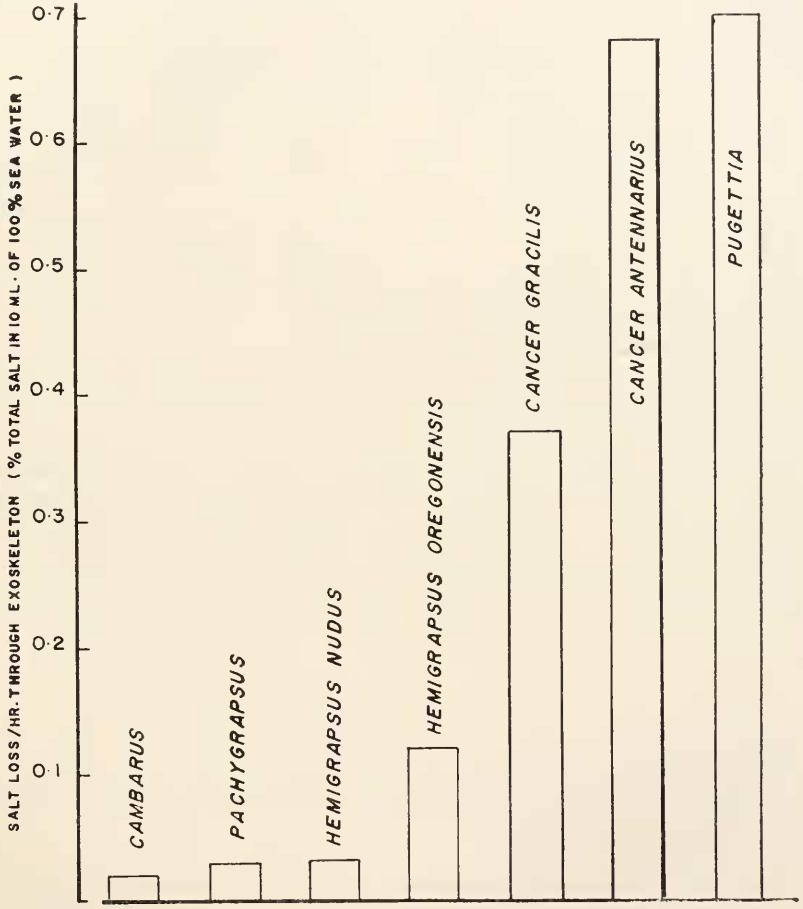

FIGURE 4. The relative permeability of exoskeleton in several decapod Crustacea. Values represent the means of three determinations for each species with a 50% sea water gradient. Variation among the three determinations for each species was in no case more than 25%.

dilute sea water if it were as permeable as *Cancer?* Much evidence is available demonstrating that animals which cannot regulate osmotically are strong ionic regulators (Prosser *et al.,* 1950). It may be that in the case of crabs, the mechanism for active, osmotic regulation is present and functioning in the non-regulators, but the permeability of the exoskeleton is so great that an osmotic gradient cannot be sustained. No evidence has been obtained on this point with respect to crabs but it

has been shown in the case of poikilosmotic sipunculids that salts can be removed from the medium without benefit of a gradient (Gross, 1954).

Osmotic regulation in the gill chamber

It is generally believed that the gills of marine crabs function as osmotic regulatory organs. Koch *et al.* (1954) have produced direct evidence in *Eriocheir* supporting this view. Gross (1955) demonstrated that the gill chamber of *Pachygrapsus* is a site of salt exchange under conditions of desiccation. As one way of testing whether this is more generally true, the salinity changes which occur in the branchial cavity of crabs removed from water were measured after the cavity was filled with water of varying salt content. It was previously shown that no significant changes occur under similar conditions when the gill chambers are filled with normal sea water (Gross, 1955).

First, the volume of fluid held in the chambers was estimated by a method reported previously (Gross, 1955). Crabs whose gill chambers had been pierced for flushing were immersed in tap water, 25% sea water or 150% sea water for a period of one hour; each cavity was then flushed out with 10 ml. of distilled water, the flushings were caught and the salinity of this fluid was measured by means of a conductivity bridge. Assuming that the gill fluid is the same concentration as the medium in which the crab was immersed and knowing the volume and salinity of the flushings, the volume of the branchial fluid can be estimated as well as the absolute quantity of salt in that fluid.

The animal was then immersed in normal sea water for about two hours to permit recovery. Again it was returned to its respective stress medium for an additional period of one hour. The crab then was removed from the water and placed in a closed container which was kept at saturated humidity by a paper towel soaked in the same medium from which the animal had been removed. Such paper was placed where the crab could not reach it. After two to seven days the animal was removed from the container and its gill chamber flushed out with distilled water, the salinity of this fluid being measured. If the latter salinity determination differed from the former for the same crab, it was suggested that salt exchanges occurred in the branchial chamber. Such was the case, for all animals with dilute branchial fluid showed an absolute salt increase after the period in the humid container, while all animals with concentrated branchial fluid showed an absolute decrease. It should be said, however, that the crabs which had been immersed in 150% sea water showed some signs of desiccation.

It is not surprising to find salt exchanges in the gill chamber, for as described above, even the chitinous exoskeleton is permeable to both salts and water. However, if it could be demonstrated that the salinity of the branchial fluid were different from that of the blood when a steady-state had been reached, then it would seem that a dynamic mechanism is present in the gill chamber, permitting an exchange of salt with the body fluids, but also being able to hold an osmotic gradient.

Now, the volume of branchial fluid for each animal was determined with the first flushing operation described above. Thus, the salinity of the second flushing will yield an estimation of the branchial fluid concentration after the period in the humid container. Results suggest that a gradient is sustained in the gill chambers of all animals which had been immersed in dilute media. The mean salinity of such

fluid from two animals which had been immersed in tap water was equivalent to 14% sea water while the blood was estimated to be greater than 75% sea water in concentration. The mean salinity in the gill chamber of four animals removed from 25% sea water was equivalent to 64% sea water while the blood concentrations were estimated to be greater than 85% sea water. Data from the specimens which had been immersed in 150% sea water were considered invalid for this aspect of the experiment.

It might be argued that the apparent gradients described above are not real, but that a great amount of branchial fluid rests on impermeable tissues. In such a case, the fluid in contact with the permeable tissues could become isotonic with the blood and this would affect the total salt content in the gill chamber only enough to give the impression that an osmotic gradient was being held. However, the large apparent gradient sustained when the branchial fluid was initially tap water, and the large salt exchange under conditions at desiccation (Gross, 1955), do not support this argument. Since all animals remained out of the water for at least two days before the salinity of their branchial fluid was measured, there was plenty of opportunity for salt exchanges to take place. It is probable, therefore, that the above described gradients are real, but the fact that more salt change occurred for animals from 25% sea water than for those from tap water raises doubt as to the quantitative value of the data, since the osmotic gradient between blood and gill chamber was greater for the tap water animals.

One more point of interest arises from this experiment. It was previously estimated that the mean branchial fluid volume for 20 *Pachygrapsus* was 1.7% body weight (Gross, 1955). The mean branchial fluid volume of those animals immersed in 150% sea water was only 0.71% body weight. Yet, again, those immersed in dilute sea water averaged 1.7% body weight. This, of course, suggests that *Pachygrapsus* has some control over the water that enters the gill chamber, which would be an aid to osmotic regulation. If such an aiding mechanism for regulation exists, it seems that it functions only for hyper-regulation, but confirmation is needed for this finding.

Metabolic work in increased osmotic stresses

It has been reported that when certain crabs are exposed to osmotic stresses, they respire more rapidly; this increase in metabolism has been interpreted as work accomplished by the regulatory mechanism (Schlieper, 1929; Schwabe, 1933; Flemister and Flemister, 1951). However, Krogh (1939) suggested that such metabolic increases are caused in part by activities of the organism other than osmotic regulation.

I have observed that certain crabs, *e.g., Pachygrapsus,* show violent attempts to escape from a medium which departs much from normal sea water in concentration. Obviously such activities would step up the entire metabolic rate of the crab, making it difficult to show the increased rate due to osmotic regulation alone.

The necessary increase in the osmo-regulatory mechanism cannot be predicted simply by knowing the normal osmotic pressure of the body fluids of an animal and the osmotic stress imposed. Rather, a knowledge of the sustained osmotic gradient is needed to give the relative amount of regulation necessary to withstand a certain stress. It is conceivable that a given amount of osmotic work will main-

tain the body fluids at viable concentrations over a range of osmotic stresses. This could be possible if the animal holds a constant gradient between blood and external medium, although the actual blood concentration alters to permit the constant gradient. Over such a range of stress, then, the constant rate of regulation would be

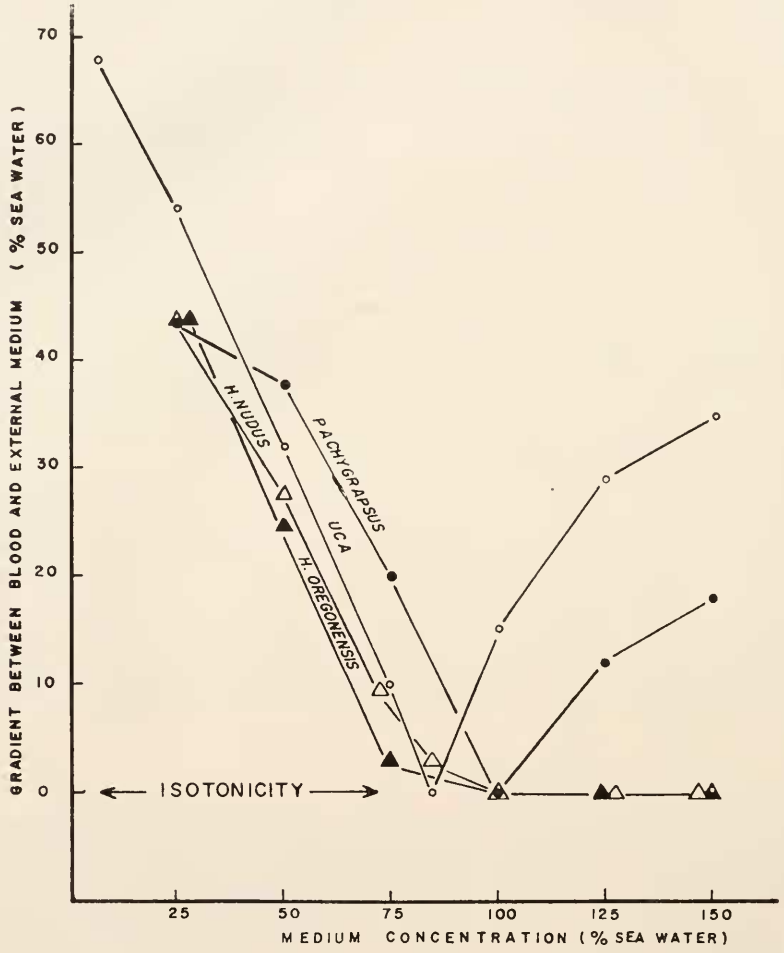

FIGURE 5. Crabs sustain greater osmotic gradients at equilibrium as greater stresses are imposed. Points are approximate mean values and for *Pachygrapsus* are calculated from the present investigation; values for *Uca, H. oregonensis,* and *H. nudus,* are calculated from Jones (1941).

expected to cause no variations in the total metabolic rate assuming constant relative roles of the various regulatory organs. On the other hand, if an increasing gradient is established more work would be expected. It has been pointed out recently by Potts (1954) that semi-permeable animals inhabiting fresh water could maintain their blood hypertonic to the medium with less work by excretion of dilute urine than by regulating at the tissue surfaces which are exposed to the external medium.

However, it was also demonstrated that such a mechanism would not be significantly advantageous over the other unless large osmotic gradients were sustained between the blood and external medium (greater than 50% sea water). The possibility should therefore be considered that at certain stresses the mechanism of regulation may shift from one process to another, and that the various processes are not neces-

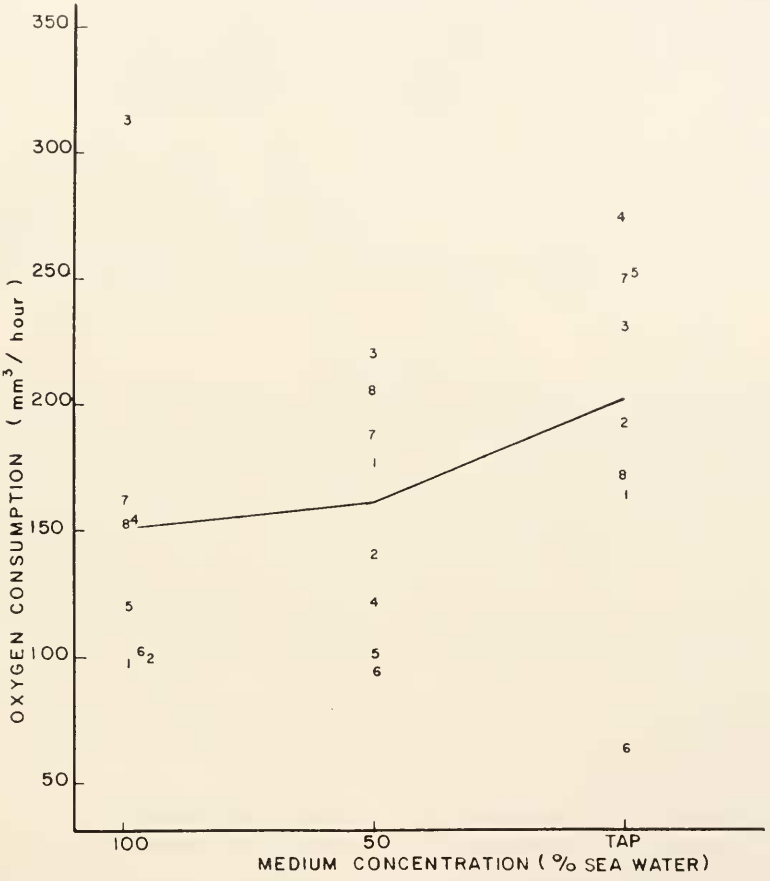

FIGURE 6. Oxygen consumption in *Uca* as a function of osmotic stress. Numerals represent individual specimens. Solid line connects mean values for all cases. Measurements made at 16° C. on crabs of approximately two grams. These could not stand out of the solution.

sarily equally efficient. Nevertheless, it does not seem to be unreasonable to assume that in general more metabolic work is required to sustain a large osmotic gradient than to sustain a small osmotic gradient.

Figure 5 demonstrates that in *Uca, Pachygrapsus, Hemigrapsus nudus,* and *H. oregonensis,* more osmotic work would be expected in greater stresses because the sustained gradients increase with the larger stresses, except for the two species of *Hemigrapsus* when they are immersed in concentrated sea water where they cannot regulate. It can be observed in *Pachygrapsus* that the sustained gradient in-

creases almost linearly with the stress, from a medium of normal sea water up to one of 50% sea water; but in 25% sea water the gradient drops off from the linear relationship with stress. This indicates that the crab must work only slightly faster, and it can be seen that with the same metabolic output an equal gradient could be held in even more dilute water. *Uca* shows almost a linear increase in the gradient up to a medium of 25% sea water and only a slight fall off from this relationship to stress in 5% sea water. *Hemigrapsus nudus* and *H. oregonensis* show behavior in dilute sea water which is somewhat different from *Pachygrapsus* and *Uca,* for they regulate weakly in 75% sea water, sustaining less than a 10% sea water gradient. With like stresses *Uca* and *Pachygrapsus* hold 22% and 20% sea water gradients, respectively. However, as the stress increases both species of *Hemigrapsus* regulate strongly, as illustrated by the increased slopes for these crabs in Figure 5.

If the additional osmotic work could manifest itself, the animals would be expected to consume more oxygen as the medium departs farther from the osmotic pressure of the body fluids. The oxygen consumption of individual specimens of *Uca* was compared when the crabs were immersed in normal sea water, 50% sea water, and in tap water. Since this species may be found commonly in normal sea water, its respiratory rate was measured first in this medium, assuming that harmful effects would be at a minimum here. For the next determination, half the animals were tested in 50% sea water, the other half in tap water. For the third determination the order was reversed so that each specimen was studied in three media. This was a control against permanent injury which might result from the heavy stress imposed by tap water. However, the order of immersion did not seem to make a difference. After determinations in 50% sea water and tap water, the crabs were placed in 100% sea water for 24 hours to allow recovery before the next determination was made. All specimens whose behavior is recorded lived in sea water for at least 48 hours after completion of the experiment. Results in Figure 6 illustrate that the metabolic rate of an individual animal does not necessarily increase with osmotic stress. In fact, of 8 animals, only numbers 2 and 7 show such a behavior. With the exception of two extreme cases, however, number 3 in normal sea water and number 6 in tap water, the respiratory rates for the group of animals seem to be higher in higher stresses. The mean of the 8 specimens shows a value for oxygen consumption in 50% sea water of about 108% of what it is in normal sea water, while in tap water it is about 135% of the value for 100% sea water.

Assuming that the normal osmotic pressure of *Uca* blood is equivalent to that of 85% sea water, there will be a 15% sea water gradient when the crab is immersed in normal sea water; in 50% sea water there will be a maximum gradient of about 35% sea water, and in tap water a maximum gradient of 85% sea water. When the animal is exposed to an osmotic stress, the amount of work should increase by the same factor as does the increased sustained gradient. When immersed in 50% sea water, regulation might increase by 35–15 or 20 units. The mean respiratory rate in 50% sea water shows 8% increase over animals immersed in normal sea water; thus, the fraction of total metabolism responsible for regulation in sea water might be calculated by simple proportion to be 6.0%. In tap water, this value is calculated to be 7.5% of the total metabolism.

These results do not agree with those of Flemister and Flemister (1951) who

studied another crab, *Ocypode*. Flemister and Flemister regarded the increased respiratory rate in hypertonic and hypotonic sea water as a manifestation of greater chloride regulation, and probably osmotic regulation. If this is the case, then about the same amount of metabolic work serves to maintain almost perfect regulation in any medium which establishes a gradient of from 25% to 50% sea water, yet in an isotonic medium, the metabolic rate is at least 20% lower. Could it be that osmotic regulation in *Ocypode* is an all-or-none process which is triggered by a salinity change in the medium? *Uca* is also a member of the family Ocypodidae, and its habitat is similar to that of *Ocypode*. It would seem questionable, that the osmo-regulatory mechanism of these closely related organisms could be essentially different.

It is this author's opinion that the interpretation of increased respiratory rates of animals subjected to osmotic stresses as a manifestation of greater osmotic work is to be questioned. This opinion is based on the inconsistent results found in individual animals in the present investigation (Fig. 6) and on the knowledge from numerous observations on a number of species that crabs are sensitive to osmotic stress and attempt to escape.

Discussion

The osmotic responses of the species studied in this investigation are not always obviously adaptive. The stenohaline forms represented by *Emerita* are presumably limited in their choice of habitat by their tissue tolerances, since they are unable to regulate. Yet, this species can tolerate salinities of from 75% sea water to 125% sea water for at least 24 hours, suggesting that the dilute waters of estuaries and the concentrated waters of tide pools might partially afford a refuge for them. It would seem to be the habits of this animal that dictate that it live near the level of the washing waves, rather than its lack of osmotic regulation.

The strong osmo-regulatory ability of *Pachygrapsus* and *Birgus* superficially seems superfluous in animals of their habits. *Pachygrapsus* generally is found in normal sea water where it does not have to regulate, or on land where it is subjected to desiccation, not direct osmotic forces. *Birgus* is found usually on dry land except for periods when it returns to the sea to reproduce. Gross (1955) discusses the significance of the ability to regulate among terrestrial and semi-terrestrial crabs, and suggests that in nature where osmotic stresses in aqueous media are rarely encountered it is of little importance as such, but appears under the artificial conditions of experimentation, *i.e.*, osmotic stresses, as a secondary manifestation of physiological processes important for life on land. It is interesting that the regulators *Pachygrapsus* and *Birgus* show large tolerances to variations in their blood concentrations.

The immediate resistance to osmotic stresses demonstrated by regulating animals may be a matter of inertia permitted by relatively impermeable exoskeletons, for the present investigation has shown a correlation between osmo-regulatory ability and integumental impermeability. The latter significantly contributes to the time factor which is important to intertidal animals which are subjected to tidal rhythms. In the case of *Hemigrapsus*, hypo-osmotic regulation for 20 hours would be adequate, because it would be rare for this animal to be exposed to the air for such a period.

Pantin (1931) has said that non-regulatory animals may posses the same mecha-

nisms permitting osmo-regulation as do regulators but to a lesser degree. It may be, in view of the above findings, that the difference between a regulator and a non-regulator could be merely a difference in permeability.

Previous investigations have produced evidence that the gills are major osmo-regulatory organs among marine crabs. The present investigation has established that a dynamic flux of salts and water occurs in the gill chamber of *Pachygrapsus*. Although no direct evidence has been produced, it is probable that the gill tissues are capable of actively transporting salts. However, the regulating mechanism may not lie entirely at a tissue level, for there is evidence suggesting that stress media can be partially excluded from the branchial chamber, therefore preventing contact with the gill tissues in large volumes.

The green glands of *Pachygrapsus* are probably not important organs of osmo-regulation, because the urine is close to the concentration of the blood in various concentrations of sea water. Prosser *et al.* (1955), however, have produced evidence that the green glands are important ion regulators. Potts (1954) has shown theoretically that a semi-permeable animal would not gain much regulatory efficiency in excreting a dilute urine, unless extreme stresses were encountered. However, the crabs used in this study are not semi-permeable. In fact, net changes in the osmotic pressure of the blood were shown to be effected mainly by salts, not water.

The relatively constant values for solute space which were calculated from the ratio of concentration changes in the blood to concentration changes in the external medium have interesting implications, namely, that if salt pools are contributing to the blood changes occurring under osmotic stress, they are doing so in a constant manner. The question remains open, whether or not a given alteration in the blood can be accounted for in the external medium.

When regulating crabs are exposed to increasing osmotic stresses, they generally sustain greater osmotic gradients. This would necessitate greater osmotic work. However, increased rates of respiration which are registered by crabs when they endure an osmotic stress cannot be interpreted as the manifestation of that increased work, for other activities, stimulated by the stress, *e.g.*, struggle to escape, cannot be isolated. It may be possible that greater osmotic work alone does not add appreciably to the total metabolic rate.

This investigation was conducted under the direction of Professor Theodore Holmes Bullock, to whom I am grateful for his most helpful encouragement and guidance. I am also indebted to Professor C. L. Prosser and Dr. J. D. Robertson for reading parts of the manuscript and offering helpful suggestions.

Summary

1. The decapod Crustacea, *Emerita, Callianassa, Upogebia, Cancer antennarius, C. gracilis* and *Pugettia* cannot regulate osmotically and from lack of tolerance are generally stenohaline.

2. *Pachygrapsus, Birgus, Hemigrapsus* and *Uca* can regulate osmotically in concentrated and dilute sea water. *Hemigrapsus* is the weakest hypo-osmotic regulator of the four species.

3. Among species where osmotic regulation occurs, it is established immediately and may be long lasting or may grow weaker with time. Although blood concentrations may fluctuate in a given stress, the phenomenon is not common.

4. Equilibrium of the blood concentration, where perfect regulation does not occur, is usually established within 24 hours following immersion; changes occasionally occur later when extreme stresses are imposed.

5. Estimates on the solute space volumes were calculated as 40% for *Emerita*, 54% for *Pachygrapsus* and about 50% for *Birgus*.

6. Concentration changes occurring in the blood of *Pachygrapsus* and *Emerita* are caused mostly by salt rather than water exchanges.

7. There is a dynamic flux of salt and water in the gill chamber of *Pachygrapsus*, thus furnishing further evidence that the gills are osmo-regulatory organs.

8. The osmotic regulating crustaceans *Cambarus, Pachygrapsus, Hemigrapsus nudus* and *H. oregonensis* have less permeable exoskeletons than the non-regulators, *Cancer gracilis, C. antennarius* and *Pugettia* by a factor of at least three.

9. *Pachygrapsus, Uca, H. nudus* and *H. oregonensis* sustain greater osmotic gradients when greater osmotic stresses are imposed. The two species of *Hemigrapsus* are weak regulators in small stress, but the osmo-regulatory activity accelerates as stresses increase. A sensitivity to absolute salinities is suggested.

10. *Uca* averages greater respiratory rates in greater osmotic stresses, but this is not necessarily so for individual specimens and the average differences are small. A discussion of the energetics of osmotic regulation reaches the conclusion that such increases in metabolism are not direct reflections of increased osmotic work but of muscular or other activity.

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